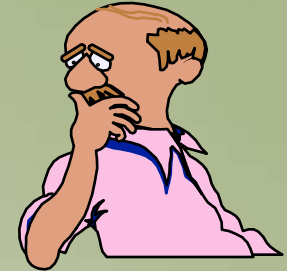


What I will say today:

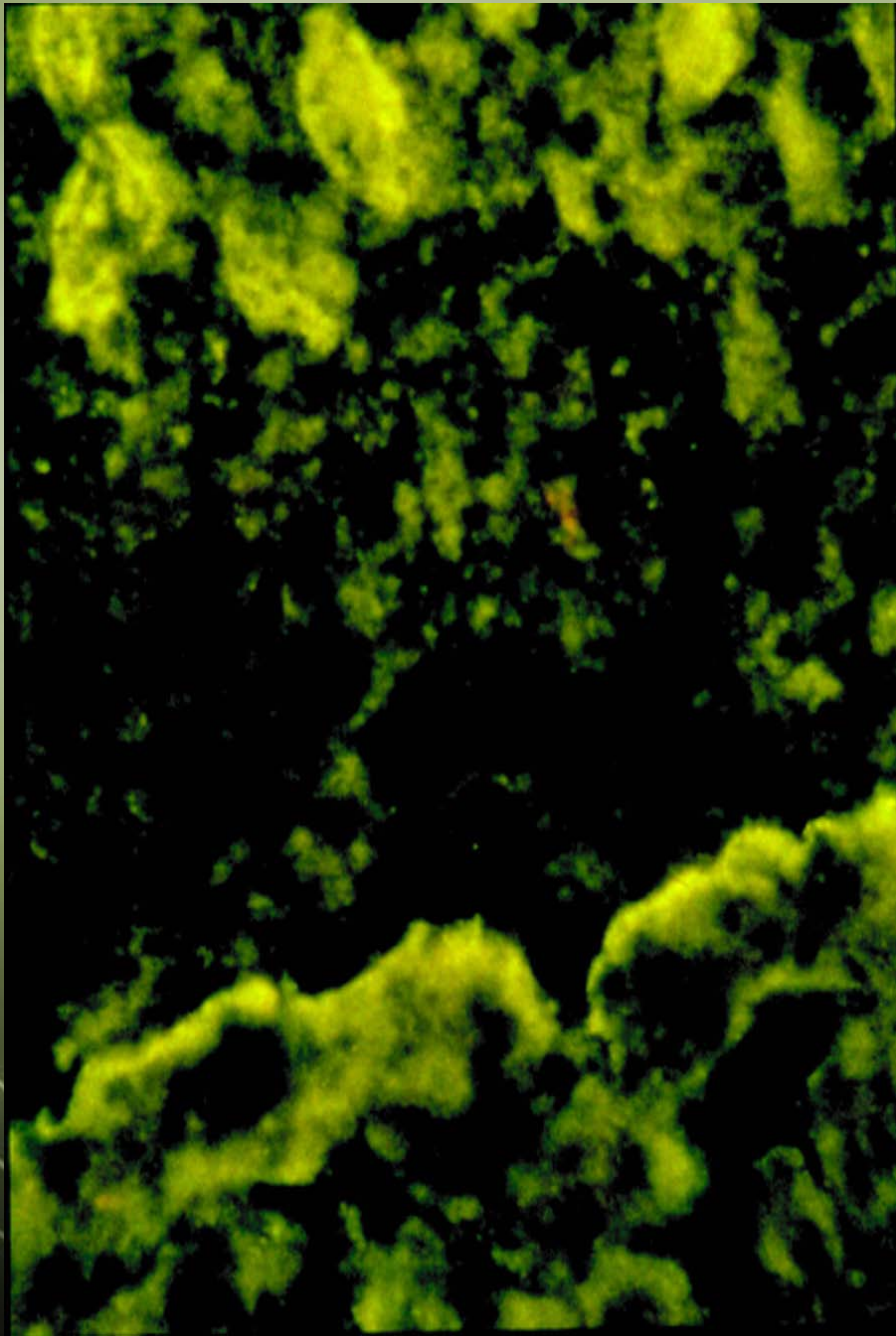
- 1. Primary biliary cirrhosis is caused
by bad genes and bad luck*
- 2. And especially by chemicals and
mimic bacteria*

Key Questions in Primary Biliary Cirrhosis

???



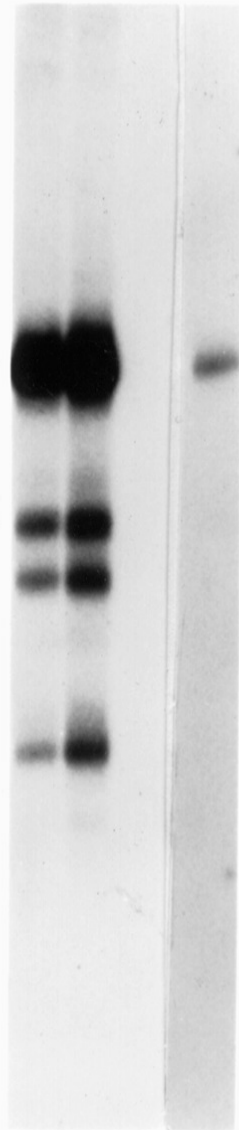
- How does the immune system initiate the immune response to self intracellular mitochondrial proteins ?
- All cells in the body have mitochondria. Then, why do only bile duct epithelial cells Become target of the self immune system ?
- Are antimitochondrial autoantibodies the primary or secondary immune response of this disease ?



A B C D

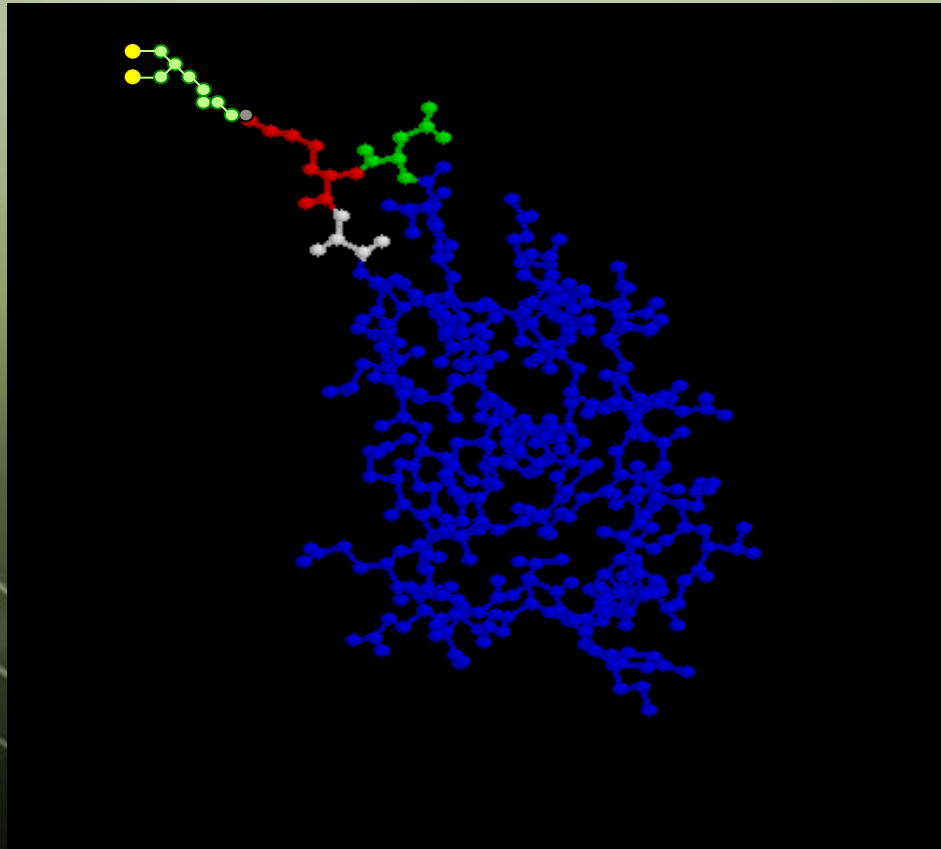
74—

52—



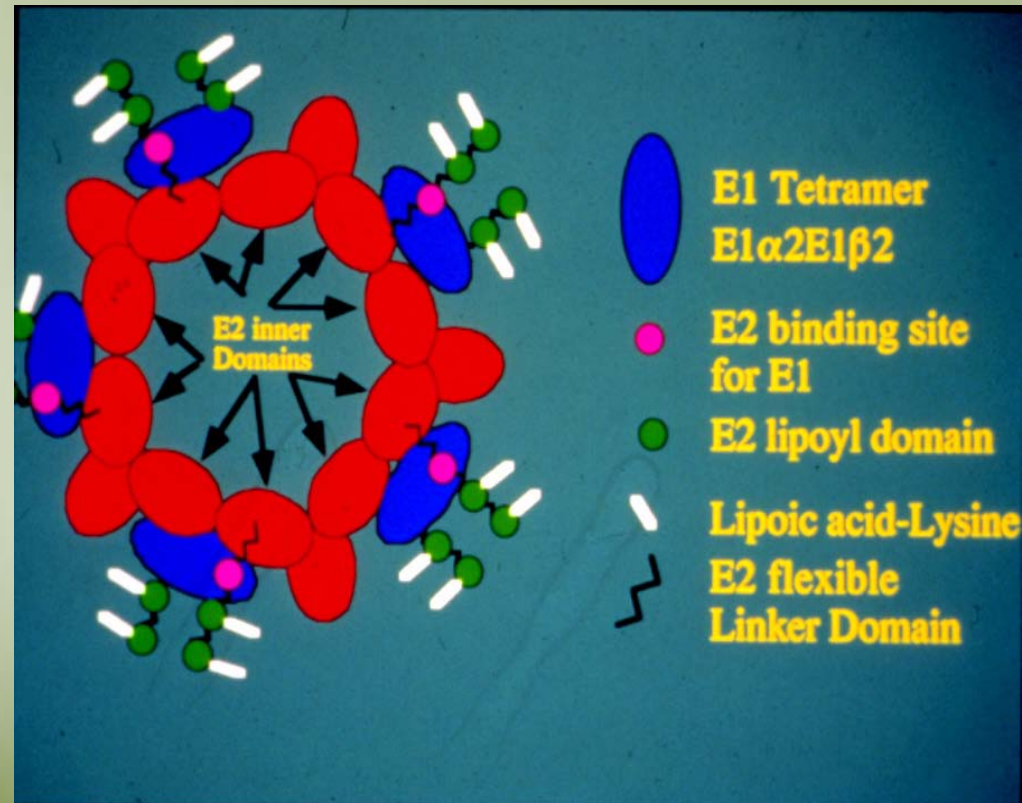
Introduction

20 years ago, Pyruvate dehydrogenase E-2 subunit (PDC-E2) was identified as the immunodominant mitochondrial autoantigen in Primary Biliary Cirrhosis (PBC)



Mitochondrial Antigens of PBC

- PDC-E2 (74 kD)
- Protein X (56 kD)
- BCOADC-E2 (52 kD)
- OGDC-E2 (48 kD)
- PDC-E1 α (39 kD)



All members of Pyruvate Dehydrogenase Complex

Result

- ⊕ If you have a positive AMA with recombinant autoantigens you better have good medical insurance

Over The years

Define
Epitopes

Environmental
(Xenobiotic Data)

Mouse
Models

1987

2007

Identified
Autoreactive
CD 4 and Cd8

Epidemiological
data

CLINICAL PRESENTATIONS

Convenient truths.

Primary biliary cirrhosis (PBC) is a autoimmune liver disease with a high female predilection.



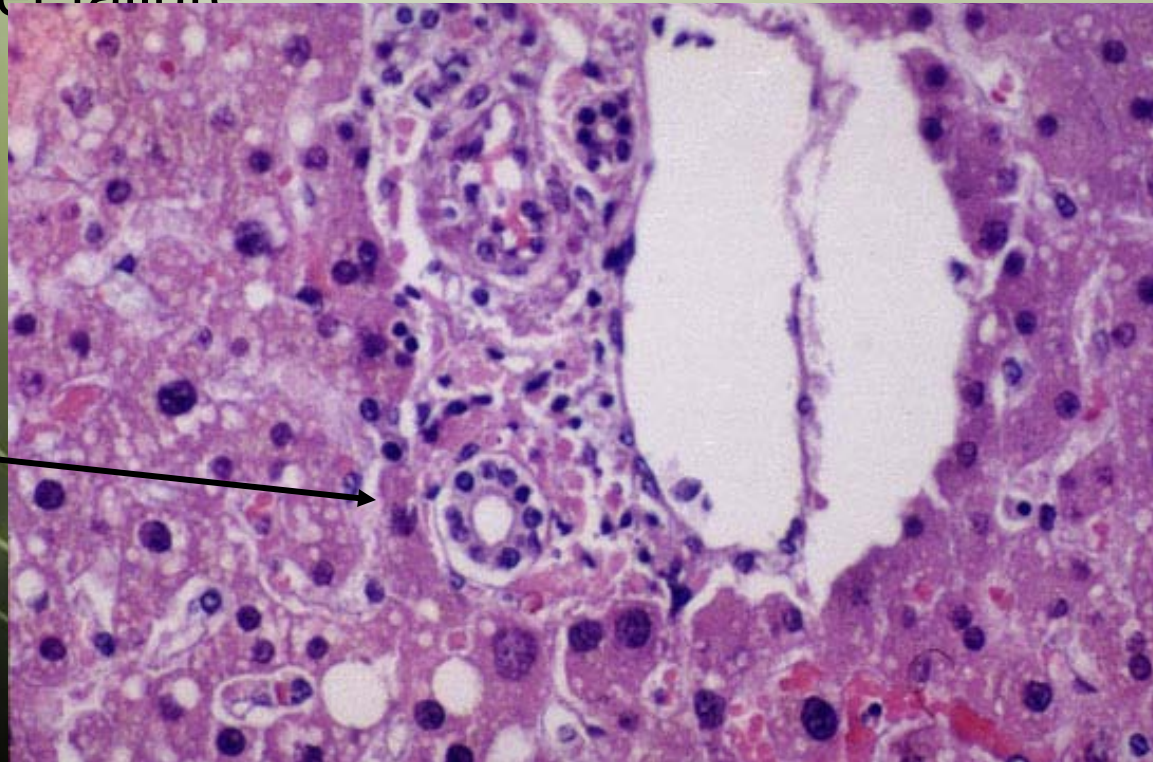
CLINICAL PRESENTATIONS



Convenient truths.

Characterized by progressive immune-mediated destruction of intrahepatic biliary ductules.

Resulting in decreased bile flow, "obstructive" liver functional indices, hepatic fibrosis and cirrhosis, and eventual liver failure



Bile Duct

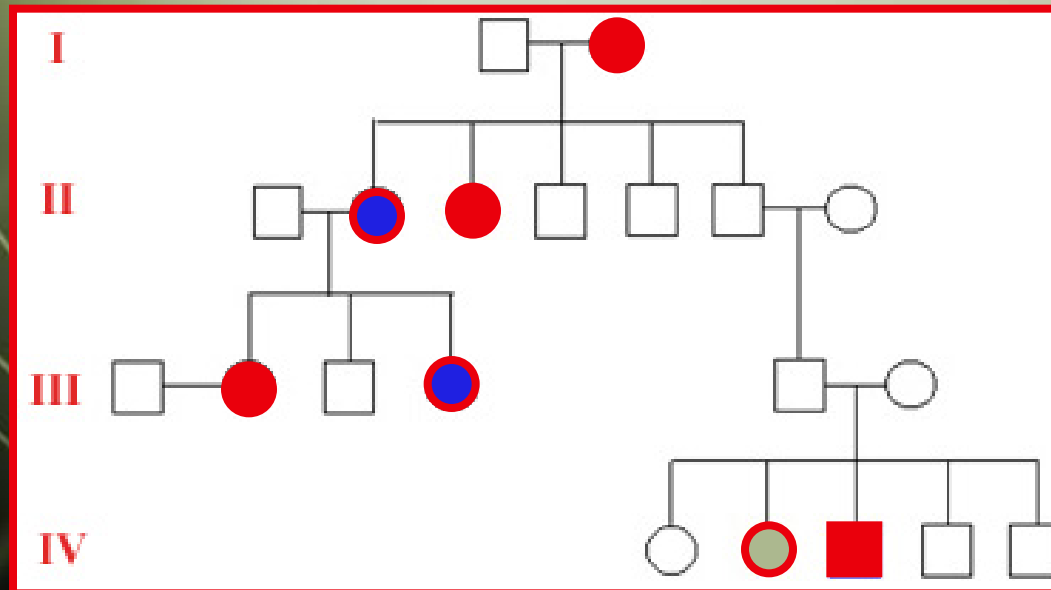


CLINICAL PRESENTATIONS



Convenient truths.

PBC is clearly associated, within individuals and among family members, with other autoimmune diseases, either organ-specific or multisystem , reflecting the “clustering” so characteristic of autoimmunity .

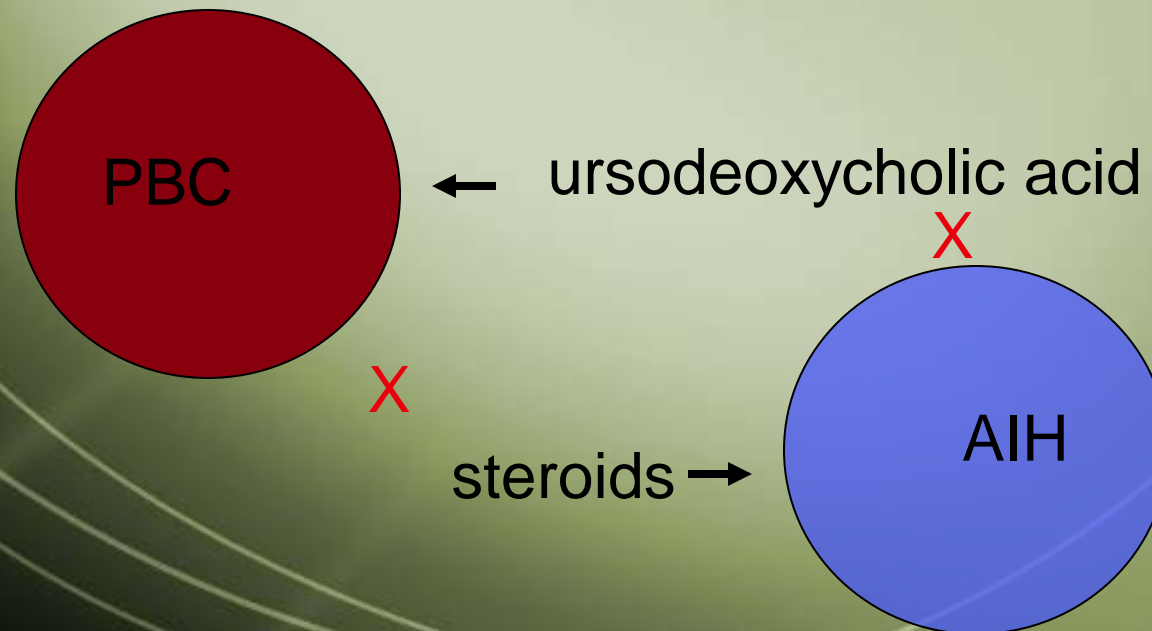


CLINICAL PRESENTATIONS

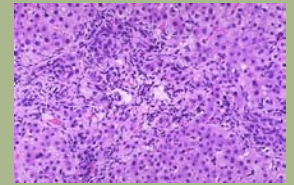


Inconvenient Truths

Additionally PBC is not highly responsive to conventional immunosuppressive drugs yet therapeutic benefit (unique to PBC) is conferred by ursodeoxycholic acid that is not regarded as an immunomodulatory or anti-inflammatory agent.

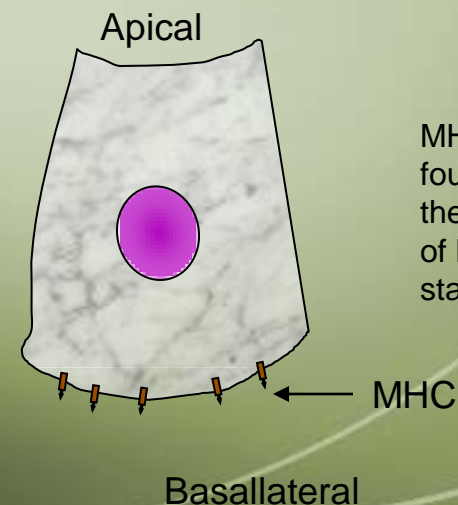
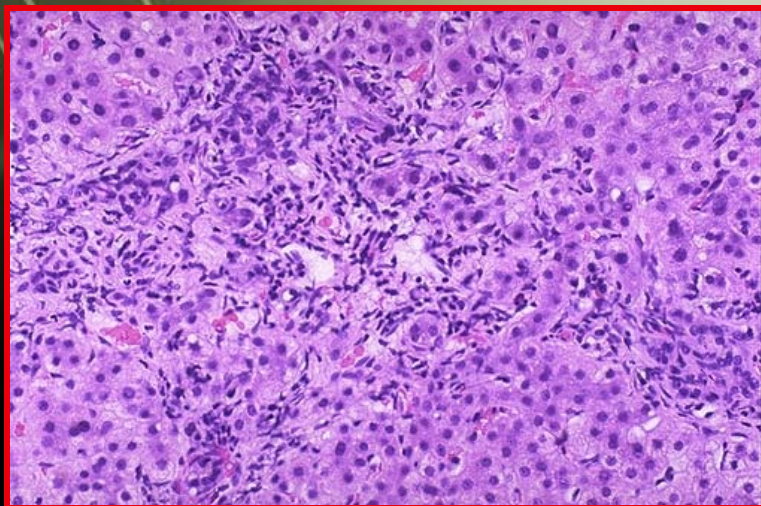


PATHOLOGIC FEATURES



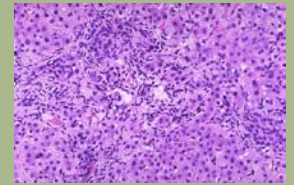
Convenient truths.

The specificity of pathological changes localized to the **bile ducts**, the presence of **lymphoid infiltration** in the portal tracts, and the readily detectable **expression of MHC antigens** on the biliary epithelium indicate that autoantigen-specific T cell responses are directed against biliary epithelial cells (BEC).



MHC molecules are found expressed on the basallateral side of BEC's in earlier stages of PBC

***Do autoreactive T cells
play a role
in the pathogenesis of
PBC?***

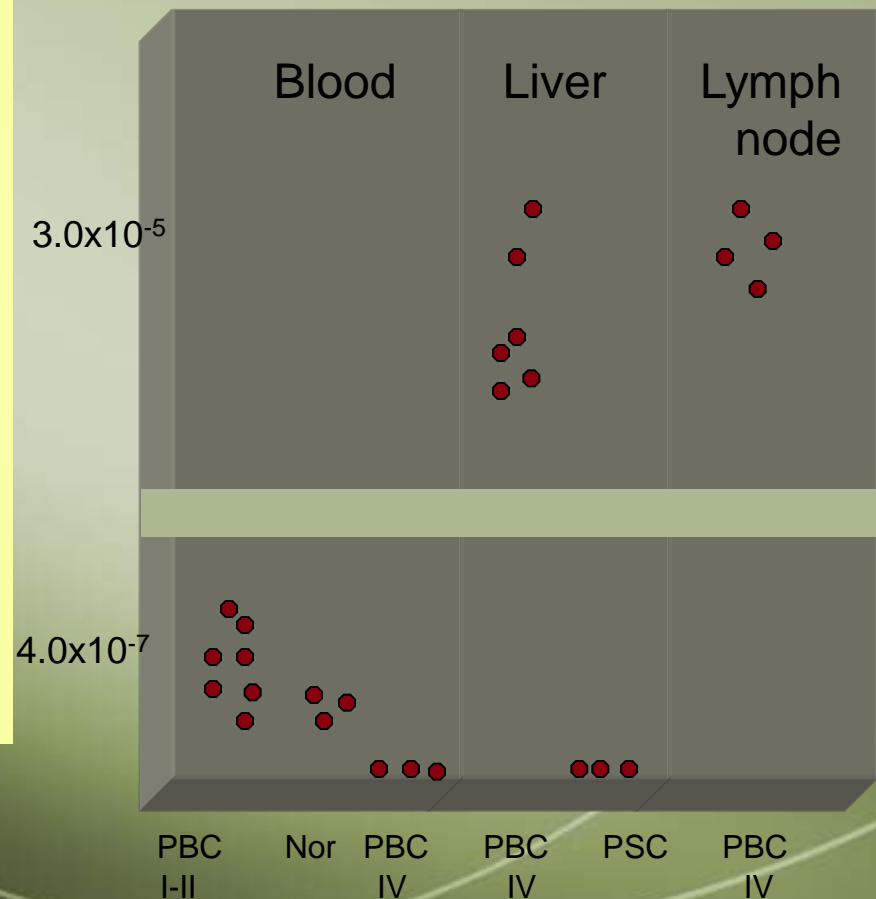


PATHOLOGIC FEATURES

Convenient truths.

Our laboratory has accumulated substantial data suggesting that the destruction of biliary cells in PBC is mediated by liver-infiltrating autoreactive **CD4+ T cells** with specificity for the immunodominant PDC-E2 autoantigen, and MHC class II-restricted target epitopes have been mapped .

Reactive frequency of T cells to inner lipoyl peptide of PDC-E2



Tetramer staining of PDC-E2 specific CD8 T cells after in vitro stimulation of PBMCs with PDC-E2₁₅₉₋₁₆₇

Sample	PDC-E2 ₁₅₉₋₁₆₇ tetramer+ CD8 ⁺ cells (%)	Sample	PDC-E2 ₁₅₉₋₁₆₇ tetramer+ CD8 ⁺ cells (%)
PBC 2	0.52	CLD*1 1	< 0.1
PBC 3	1.13	CLD 2	< 0.1
PBC 5	0.53	CLD 3	< 0.1
PBC 8	0.13	CLD 4	< 0.1
PBC 9	0.14	Normal 1	< 0.1
PBC 10	0.24	Normal 2	< 0.1
PBC 13	0.82	Normal 3	< 0.1
PBC 14	0.36	Normal 4	< 0.1
PBC 15	0.66	A2 neg PBC*2 25	< 0.1
PBC 16	0.27	A2 neg PBC 26	< 0.1
PBC 17	0.12	A2 neg PBC 27	< 0.1
PBC 18	1.20	A2 neg PBC 28	< 0.1
Mean ± SD	0.42 ± 0.33*3		

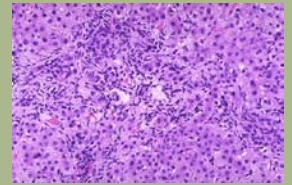
*1: CLD = chronic liver diseases controls

*2: A2 neg PBC = HLA A2 negative PBC

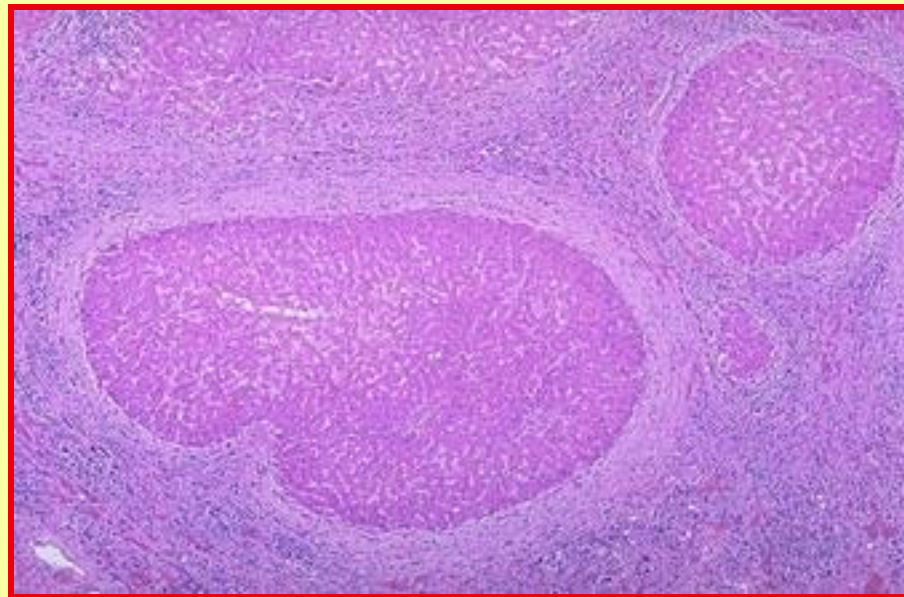
*3: Values for PBC patients were significantly (P<0.0001) higher than those from controls (CLD, Normals, and A2 neg PBC).

PATHOLOGIC FEATURES

Inconvenient Truths



In early histologic lesions there is eosinophilia, and also granulomas that are unique to PBC versus other liver



disease except for sarcoidosis , and are unique versus autoimmune pathologies overall. Granulomas have led to suspicions of an microbial basis for PBC , but this has not been established, nor can retroviral infection be substantiated .

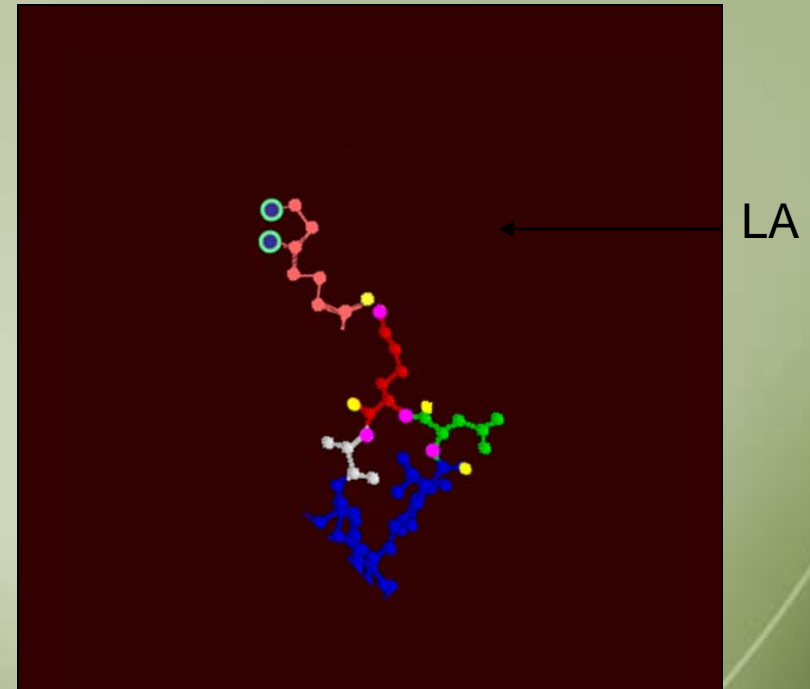
Immunologic features:

Convenient truths.

2-oxo-acid dehydrogenase complexes (2-OADC)

The autoantigens include the E2 subunits of the pyruvate dehydrogenase complex (PDC-E2), the branched chain 2-oxo-acid dehydrogenase complex (BCOADC-E2), the 2-oxo-glutaric acid dehydrogenase complex (OGDC-E2), and additionally the dihydrolipoamide dehydrogenase binding protein (E3BP) . All of these protein have at least one lipoyl domain (lipoic acid) (LA).

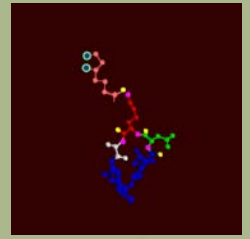
LA is exposed on PDC-E2, swings about, and is a ideal target.



Highly homologous proteins are found throughout the bacterial world.

Immunologic features:

Convenient truths.



Overlapping epitopes

Human PDC-E2

B cell ***KVGEKLSEGDLLAEIETDKATIGFEVQEEGY***

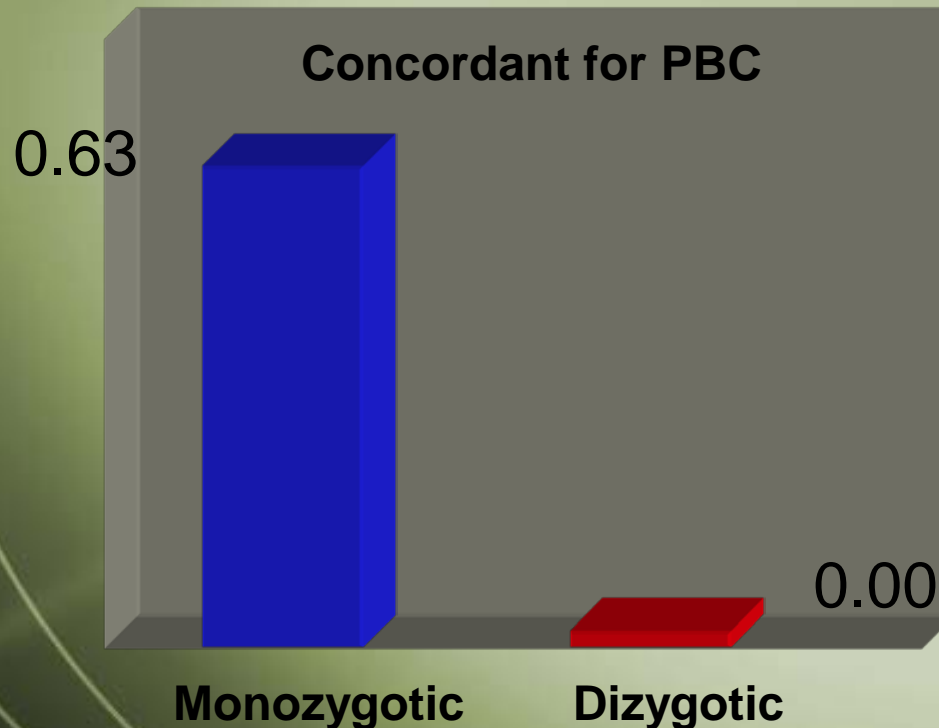
CD4 KVGEKLSE ***GDLLAEIETDKATIGFEVQEEGY***

CD8 KVGE ***KLSEGDLLAEIETDKATIGFEVQEEGY***

Assays using these epitopes have found **100-150 fold** increase in the number of autoreactive CD4+ T cells and a **10 fold** increase in the number of autoreactive CD8+ T cell found in the liver compared to the peripheral blood.

GENETIC INFLUENCES

Convenient truths.



In **5 of 8 sets of the monozygotic twins**, both individuals had PBC (0.63 concordance) but among the **8 dizygotic twin** pairs, **none** were concordant for PBC. Interestingly, the age at onset of disease was similar in 4 of the 5 concordant monozygotic twin pairs. Hence, while the concordance rate of PBC in identical twins is among the highest reported for any autoimmune disease, some discordant pairs were identified.



ENVIRONMENTAL INFLUENCES

Convenient truths

From our epidemiology study we found an association with;

History of urinary tract infections,
Past cigarette smoking,
Use of reproductive hormone replacement
Frequent use of nail polish.

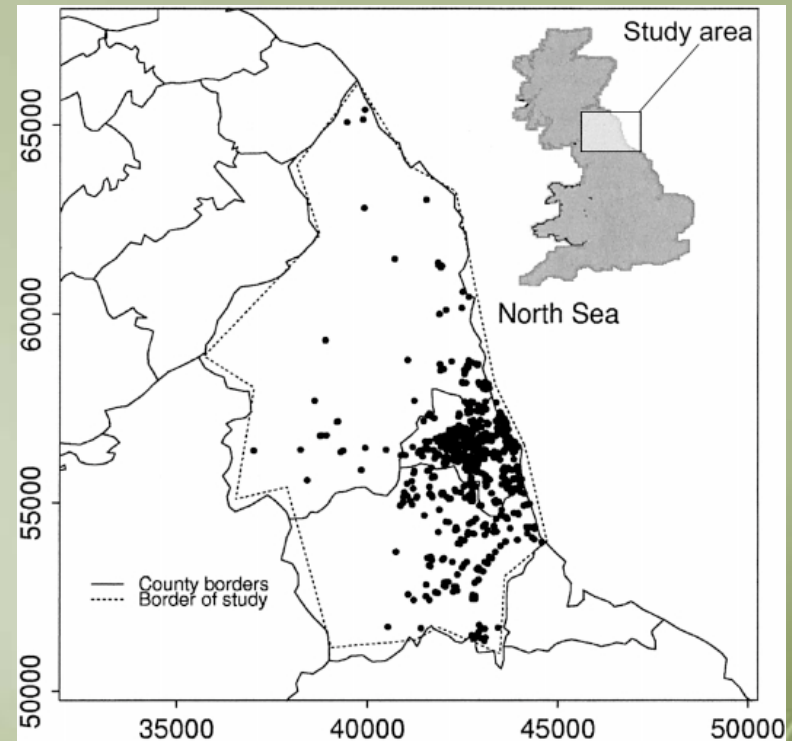
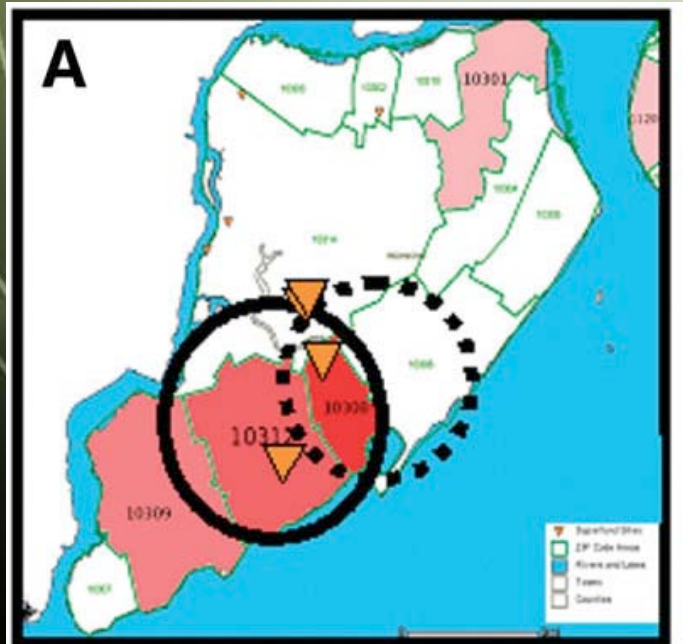


ENVIRONMENTAL INFLUENCES



Convenient truths

Toxic Superfund site
clustering of PBC in New
York



Clustering of PBC
cases in Northern UK

ENVIRONMENTAL INFLUENCES

Convenient truths

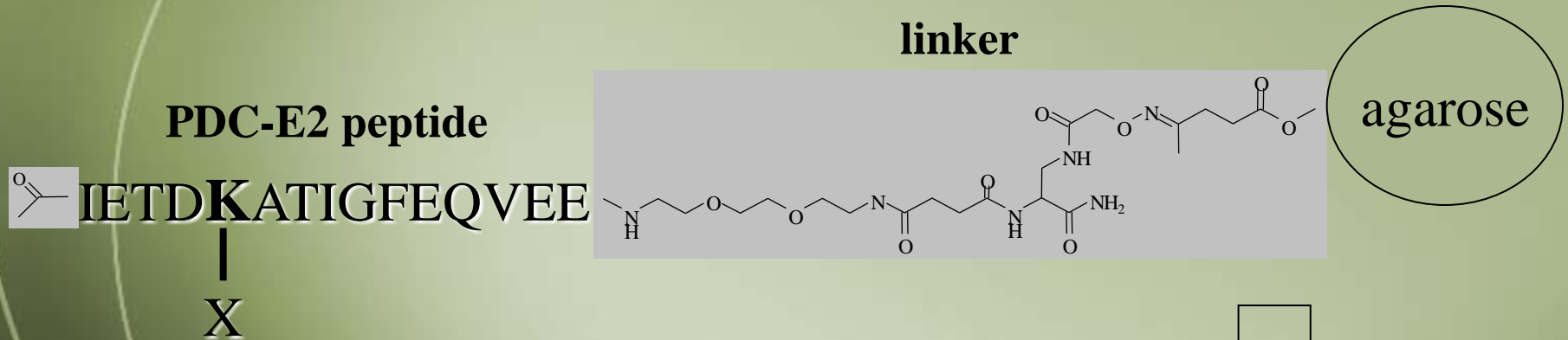


Million Tons of man made chemicals



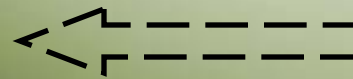
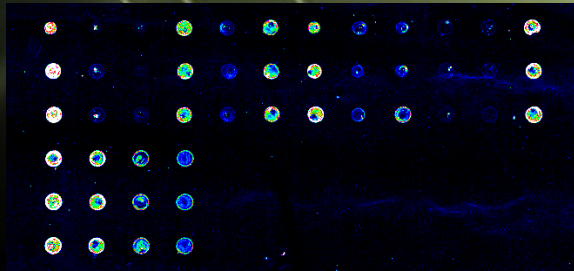
~100,000 existing chemicals

Schematic of Peptide-Xenobiotic Microarray

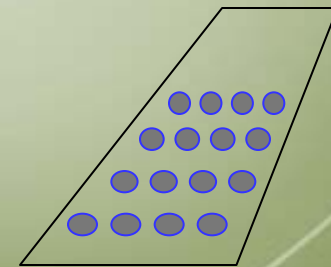


X: xenobiotic mimeotope

Spot onto microarray
glass slides

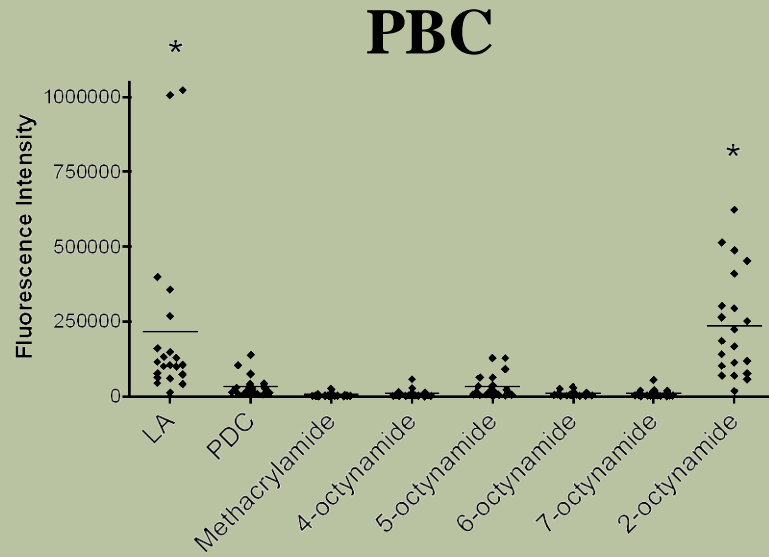


- 1) Block
- 2) Serum
- 3) Detection Ab (cy3/5-anti-IgG/M)
- 4) Dry & scan

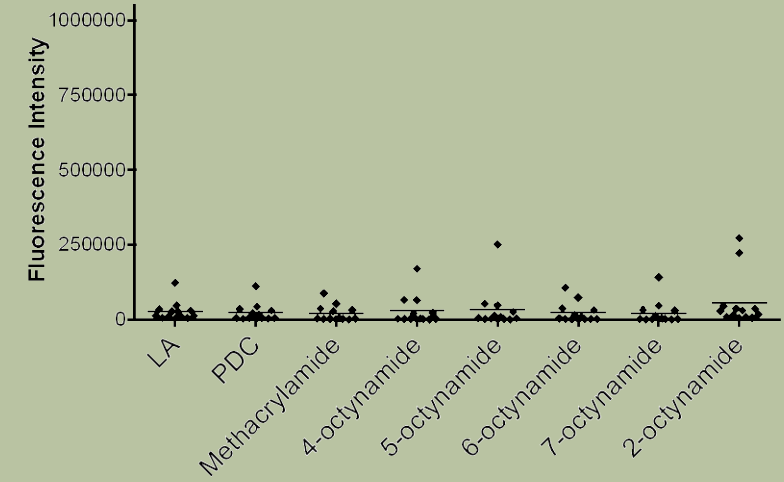


Reactivity of n-octynamides with PBC sera

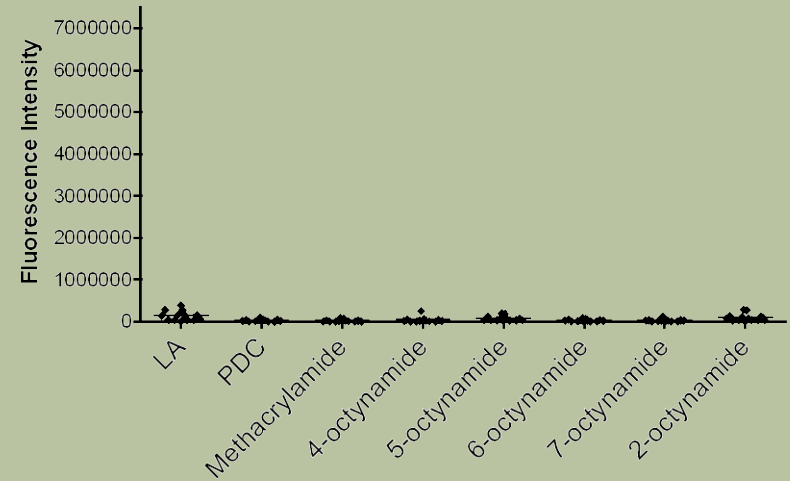
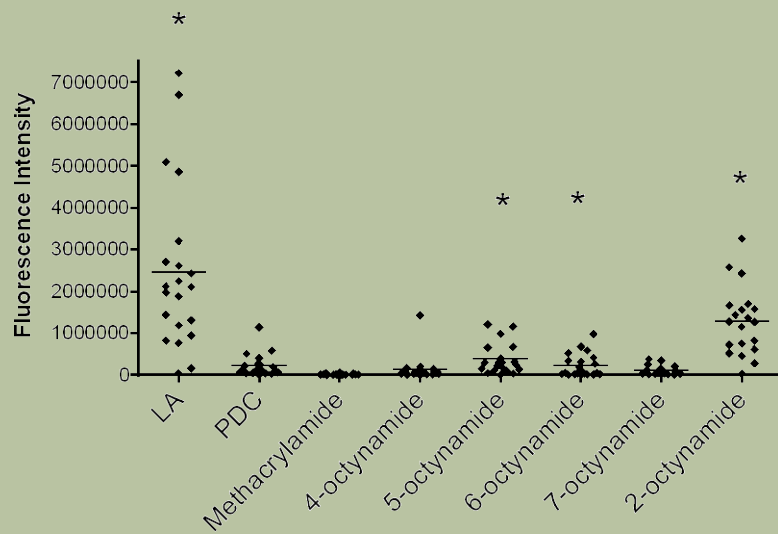
IgG



Control

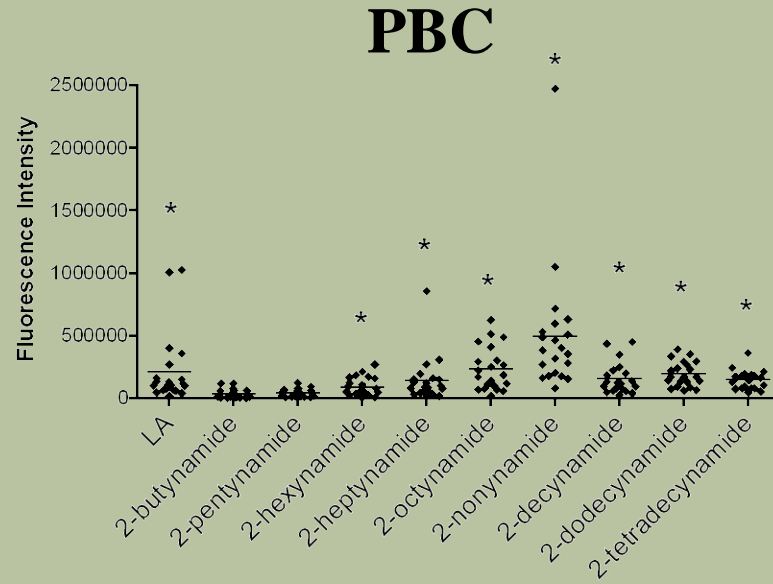


IgM

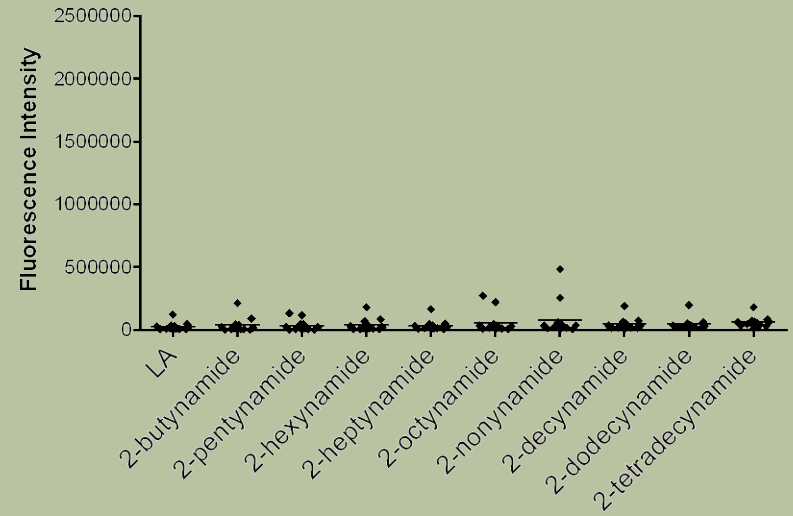


Reactivity of 2-alkynamides with PBC sera

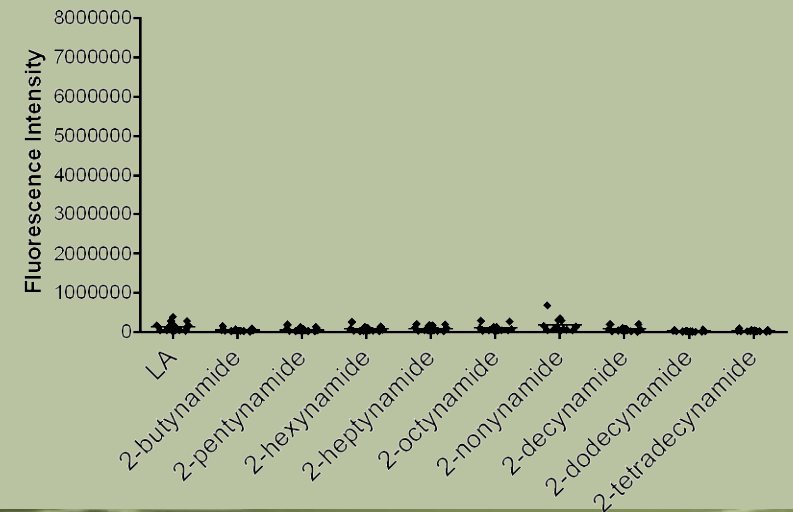
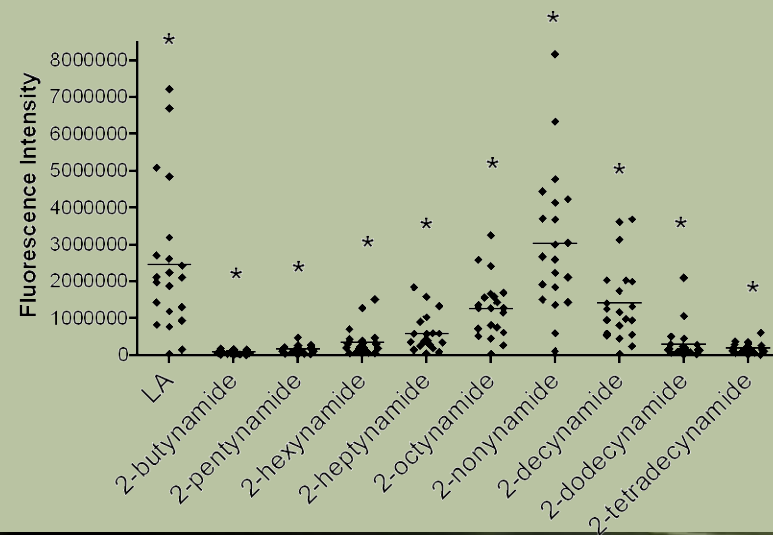
IgG



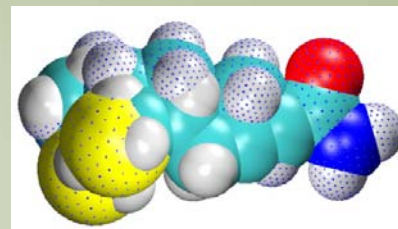
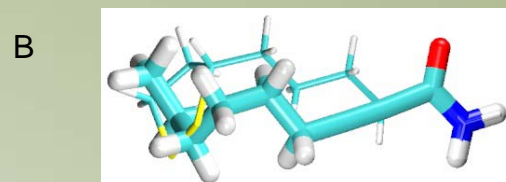
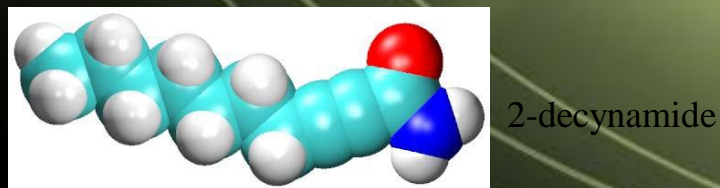
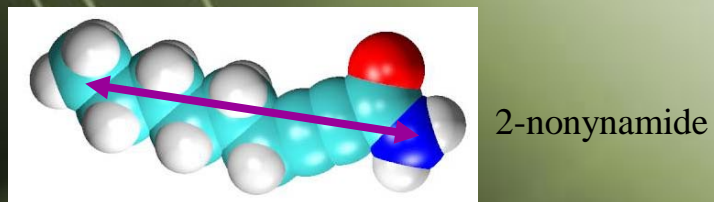
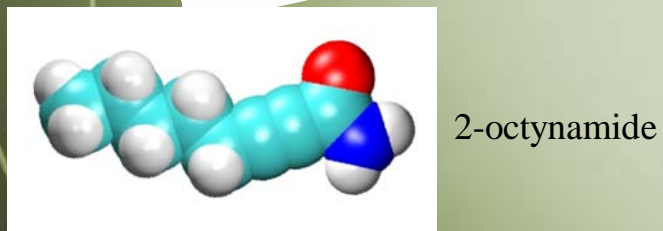
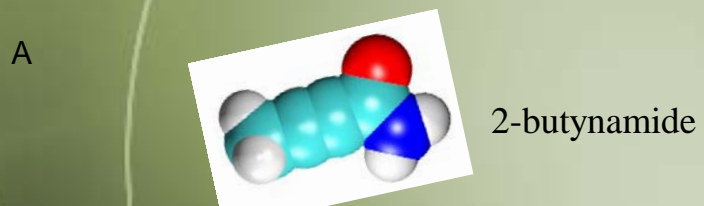
Control



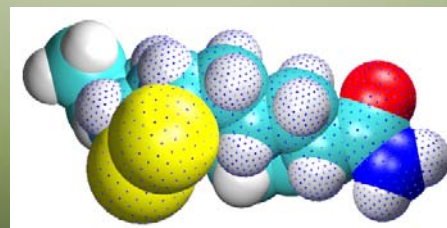
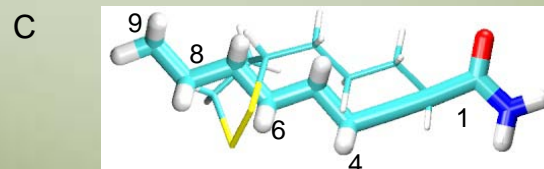
IgM



Molecular mimicry between lipoamide and 2-nonynamide



lipoamide vs.
2-nonynamide,
corkscrew
conformation



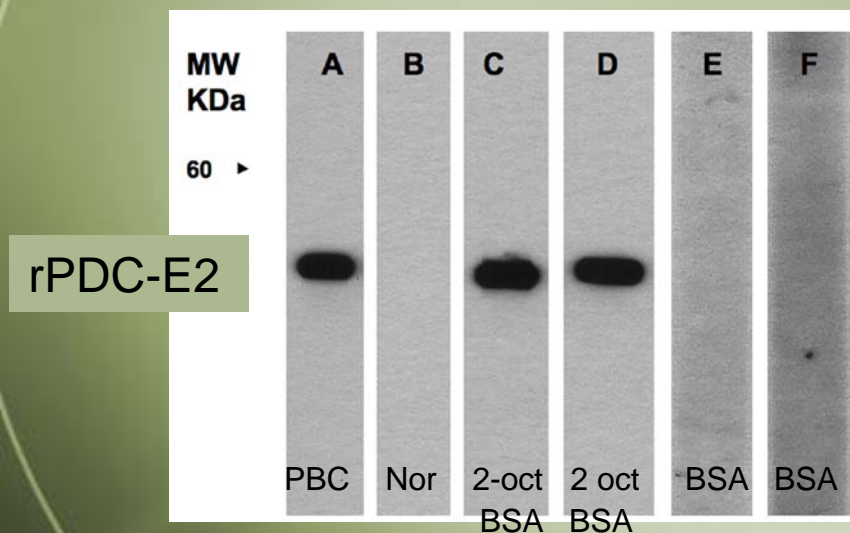
lipoamide vs.
2-nonynamide,
extended
conformation

ENVIRONMENTAL INFLUENCES

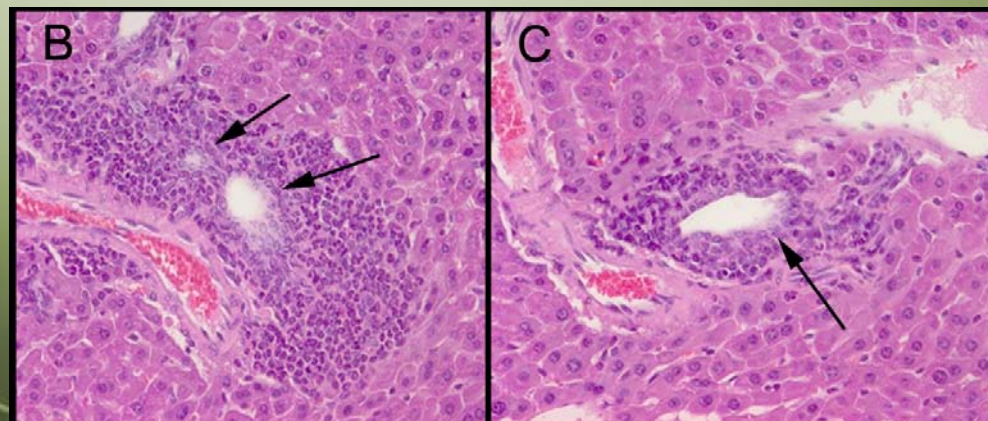


Convenient truths

Xenobiotics in mice



AMA of 2-octonyoic -
BSA immunized mice



Yuki Morotoki and the Mouse Group Family at UC Davis-Gershwin Lab



dnTGFβRII

- **Lack or aberrant activity of TGF-β signaling contributes to a loss of self tolerance to autoantigenic proteins in the liver, which in turn leads to autoimmunity and more toward activation of an intrinsically self-reactive T cell repertoire in which necessary regulatory T cell (T reg) influences are lacking**
- **dnTGFβRII mouse is an animal model in which immunoregulatory defects within the lymphocytic and phagocytic components of the immune system, potentially in association with a particular vulnerability of the biliary ductular target tissue, initiate an autoimmune response resembling that seen in PBC**

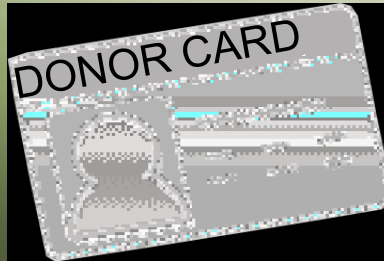
Convenient truths

Currently, more than 20,000 people in the United States are waiting for liver transplants.

About 6000 liver transplantations were performed in the United States last year.

Inconvenient truth.

About 2000 of these people will die waiting for a transplant.



?